(12) PATENT (11) Application No. AU 199963469 B2
(19) AUSTRALIAN PATENT OFFICE (10) Patent No. 764016

(54) Title
Capsules with porous mineral cortex

(51)[6] International Patent Classification(s)
A61K 009/50

(21) Application No: 199963469 (22) Application Date: 1999.10.27

(87) WIPO No: WO00/25761

(30) Priority Data

(31) Number (32) Date (33) Country
98/13695 1998.10.30 FR

(43) Publication Date : 2000.05.22
(43) Publication Journal Date : 2000.07.20
(44) Accepted Journal Date : 2003.08.07

(71) Applicant(s)
Rhodia Chimie

(72) Inventor(s)
Dominique Dupuis; Catherine Jourdat

(74) Agent/Attorney
Baldwin Shelston Waters,Level 21,60 Margaret Street,SYDNEY NSW 2000

(56) Related Art
AU 87843/98

6 5469/99

PCT ORGANISATION MONDIALE DE LA PROPRIETE INTELLECTUELLE
Bureau international

DEMANDE INTERNATIONALE PUBLIEE EN VERTU DU TRAITE DE COOPERATION EN MATIERE DE BREVETS (PCT)

(51) Classification internationale des brevets 7 : A61K 9/50 — A1

(11) Numéro de publication internationale: WO 00/25761

(43) Date de publication internationale: 11 mai 2000 (11.05.00)

(21) Numéro de la demande internationale: PCT/FR99/02614

(22) Date de dépôt international: 27 octobre 1999 (27.10.99)

(30) Données relatives à la priorité:
98/13695 30 octobre 1998 (30.10.98) FR

(71) Déposant (*pour tous les Etats désignés sauf US*): RHODIA CHIMIE [FR/FR]; ~~25, quai Paul Doumer, F-92408 Courbevoie Cedex (FR).~~ 26 quai Alphonse Le Gallo, 92512, Boulogne-Billancourt Cedex France

(72) Inventeurs; et
(75) Inventeurs/Déposants (*US seulement*): DUPUIS, Dominique [FR/FR]; Appartement 40, 60, rue Galliéni, F-95170 Deuil-la-Barre (FR). JOURDAT, Catherine [FR/FR]; 4, Chemin du Vallon, F-69110 Sainte-Foy-Lès-Lyon (FR).

(74) Mandataire: DUBRUC, Philippe; Rhodia Services, Direction de la Propriété Industrielle, 25, quai Paul Doumer, F-92408 Courbevoie Cedex (FR).

(81) Etats désignés: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, brevet ARIPO (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), brevet eurasien (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), brevet européen (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), brevet OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Publiée
Avec rapport de recherche internationale.

AUSTRALIAN SEC 104 PATENT OFFICE

(54) Title: CAPSULES WITH POROUS MINERAL CORTEX

(54) Titre: CAPSULES A ECORCE MINERALE POREUSE

(57) Abstract

The invention concerns a mineral capsule consisting of a mineral cortex and a liquid core wherein is immobilised at least an active biological material. The invention also concerns a method for preparing said capsules and their uses.

(57) Abrégé

L'invention a pour objet une capsule minérale constituée d'une écorce minérale et d'un noyau liquide dans lequel est immobilisée, au moins une matière active biologique. Elle se rapporte en outre à un procédé de préparation desdites capsules ainsi qu'à leurs utilisations.

Capsules with a porous inorganic shell

The present invention relates to novel capsules with a porous inorganic shell, in which one or more active biological substances is (are) immobilized in a liquid medium.

Any discussion of the prior art throughout the specification should in no way be considered as an admission that such prior art is widely known or forms part of common general knowledge in the field.

In general, techniques for immobilizing active substances are directed toward limiting and more preferably blocking the free migration thereof into a surrounding medium.

There are two general immobilization methods:

The first method consists in attaching the active substance to be immobilized to a surface of support type, either by adsorption (interactions of ionic type, Van der Waals attachments), or by covalent attachment or via an intermediate compound.

The second method is directed, on the other hand, toward physically retaining the active substance with a solid or porous matrix such as a stabilized gel.

The second approach is widely used for immobilizing active biological substances such as cells or enzymes, for example. The active biological substances under consideration are most commonly physically encapsulated within a polymeric matrix.

The encapsulation substance should, in fact, satisfy several requirements:

- it should contribute toward ensuring the stability of the structure and the activity of the active substance immobilized;
- it should have a porosity sufficient to control the diffusion of the active substance immobilized and, where appropriate, the exchanges thereof with the surrounding medium, and
- it should allow, where appropriate, easy re-use of the active substance immobilized.

The substances most commonly used for carrying out this type of immobilization are hydrocolloid gels. By way of illustration of these gels, mention may be made, in particular, of natural gels such as alginates and carrageenans. However, other polymeric lattices of synthetic nature have also been developed, such as that based on polyacrylamide.

This type of encapsulation is generally obtained by adding the active substance to be immobilized, in the form of a suspension, to an aqueous solution of a precursor of the encapsulation substance. The precursor solution is then transformed therein into droplets, generally by dispersion. Finally, these droplets are stabilized in the form of beads in which the active substance is trapped, either by polymerization or any other type of crosslinking.

It has also been proposed to encapsulate enzymes in a porous structure of inorganic nature. The

encapsulation technique selected is then related to the sol-gel technique. According to this encapsulation method, the hydrolysis and polycondensation of a metal alkoxide is initiated in water or in aqueous-alcoholic medium, the enzyme is dispersed therein, and the resulting composition is subsequently gelled and then dried.

Compared to organic matrices based on a natural gel of alginate or carrageenan type, these inorganic matrices are particularly advantageous. Their mechanical resistance thereof is considerably increased. They generally have a hydrophilic nature, and also better solvent- and pH-stability. Furthermore, they are stable in concentrated saline medium, unlike the organic matrices based on alginate, for example.

However, just like the organic matrix encapsulation systems, these inorganic matrices are unsuitable for packaging active biological substances which need to be conserved in a liquid medium. Moreover, the low porosity of these systems, due to the matrixial nature thereof, constitutes an obstacle to the growth of active biological substances and to the maintenance of the activity thereof.

It is an object of the present invention to overcome or ameliorate at least one of the disadvantages of the prior art, or to provide a useful alternative.

According to a first aspect, the present invention provides a process for preparing capsules according to the first aspect, said process comprising

1) emulsifying a liquid medium containing at least one active biological substance, in a second phase which is immiscible with said liquid medium, so as to disperse it therein in the form of droplets,

2) bringing into contact, in the emulsion thus obtained, a hydrolyzable and polycondensable silicon, under temperature and pH conditions conducive to the formation of a precipitate consisting of the corresponding oxide or hydroxide, and

3) recovering the inorganic capsules thus formed and, where appropriate, purifying them, wherein the formation of the inorganic precipitate in the second step is carried out in the presence of an amphiphilic surfactant system present in the emulsion and capable of concentrating the deposit of the inorganic particles of said precipitate formed at the interface of the droplets and the second phase, and of effectively blocking their diffusion in said droplets.

According to a second aspect, the present invention provides a process for preparing capsules according to the second aspect, said process comprising

1) emulsifying a liquid medium containing at least one active biological substance, in a second

phase which is immiscible with said liquid medium, so as to disperse it therein in the form of droplets,

2) bringing into contact, in the emulsion thus obtained, at least one hydrolyzable and polycondesable zirconium, aluminium and/or transition metal compound, under temperature and pH conditions conducive to the formation of a precipitate consisting of the corresponding oxide or hydroxide, and

3) recovering the inorganic capsules thus formed and, where appropriate, purifying them, wherein the formation of the inorganic precipitate in the second step is carried out in the presence of an amphiphilic surfactant system present in the emulsion and capable of concentrating the deposit of the inorganic particles of said precipitate formed at the interface of the droplets and the second phase, and of effectively blocking their diffusion in said droplets.

Unless the context clearly requires otherwise, throughout the description and the claims, the words 'comprise', 'comprising', 'consisting' and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of "including, but not limited to".

For the purpose of the present invention, the name "active biological substance" is intended to cover any molecule, cell or organism of industrial value due to the biological activity thereof.

By way of illustration of these active biological substances, mention may be made in particular of cellular organisms, for instance microorganisms such as bacteria, yeasts, fungi and algae, cells of animal or plant origin, enzymes or proteins such as antibodies, for example.

They are more preferably living organisms or cells.

As examples of biologically active molecules, mention may be made more particularly of enzymes such as hydrolases, nucleases, oxidases, proteases, isomerases and analogs.

They may, in particular, be oxidoreductases such as alcohol dehydrogenases, oxygenases and glucose dehydrogenases, transferases such as D-glutamyl transferase, lyases such as fumarase and aspartase, hydrolases such as lipases, nitrile hydratases, lactases and acylases, and also isomerases. They may also be proteins or protein complexes of the cytochrome C, hemoglobin, myoglobin, transferrin or superoxide dismutase type, or antibodies.

As examples of bacteria, mention may be made more particularly of lactic acid bacteria and environmental bacteria.

Unlike the conventional encapsulation systems mentioned above, the active biological substance is, in the case of the present invention, not absorbed in the dispersed form in a matrix, but concentrated in a liquid medium which is isolated from the surrounding medium by an inorganic shell.

For the purpose of the present invention, the term "liquid medium" is intended to mean a medium capable of ensuring the conservation of the activity and structure and/or the survival and, where appropriate, the internal development of an active biological substance, this medium being in a fluid form such as a liquid. As such, it differs from the media of alginate or carrageenan type which, themselves, are related to media in the form of a shell.

It may in particular be the natural biological medium of the active biological substance immobilized. Generally, this liquid biological medium is or derives from an aqueous medium.

Of course, this liquid medium can be buffered and/or supplemented with trace elements, sugars, salts and any other nutrient agent which may be required for the conservation of the activity and the structure and/or the survival and, where appropriate, the internal development of the active biological substance immobilized.

These active substances, depending on their water-soluble nature, are either solubilized or dispersed in the liquid medium.

The inorganic shell obtained according to the process of the invention has the double advantage of effectively protecting the liquid medium and the active substance(s) which it contains and, where appropriate, of allowing exchanges thereof with the surrounding medium of the capsules. This is in particular carried out by adjusting the degree of porosity of the inorganic capsules obtained according to the invention.

According to a first variant of the invention, the inorganic capsules obtained can be nonporous. This specificity is more particularly advantageous when the intention is essentially to ensure effective protection of the liquid medium,

incorporating at least one active biological substance, with respect to its surrounding medium. Unlike the microcapsules which are obtained by interfacial polycondensation and which consist of an inorganic organic? shell, the capsules obtained according to the invention are much more mechanically, chemically and heat resistant due to the inorganic nature of their shell. In this particular case, the aqueous medium and, where appropriate, the active substance which it contains are released generally by fractionation of the capsule or by induced degradation thereof.

According to a second variant of the invention, the capsules obtained can be porous and this porosity can be controlled. This has a significant advantage when the intention is, besides protecting the liquid medium and the active substance which it contains, to allow exchanges thereof with the surrounding medium of said capsules.

In fact, this adjustment of the porosity and similarly that of the size of the capsules are accomplished through mainly the choice of the inorganic substance constituting the inorganic shell or, more precisely, the choice of the precursor of this substance. This aspect of the invention is discussed in greater detail hereinafter.

The present invention is therefore particularly advantageous insofar as it provides a

reliable packaging method which is compatible with the internal development of the active substances immobilized, and which can be modulated as a function of the nature and the amount of active substance(s) immobilized.

The preservation in the capsule of a liquid medium which can be the natural biological medium of the active substance immobilized guarantees sustained functioning of said active substance, this functioning possibly, for example, causing a production of metabolites or being enzymatic.

The inorganic nature of the shell of the capsules claimed constitutes an effective protective barrier for the active biological substance with respect to the surrounding medium, while at the same time allowing, where appropriate, exchanges thereof with this medium.

The size of the capsules is controllable, it can be adjusted as a function of the constraints linked to the size of the active substances to be encapsulated or to the number of these active biological substances.

Finally, in the context of the present invention, this capsule is prepared under operating conditions which are sufficiently gentle so as not to affect the integrity of said active substances. The active substance to be immobilized is not in fact

exposed, while it is packaged, to temperature and, where appropriate, pH values likely to harm it.

With regard to the composition of the inorganic shell, it consists of at least one aluminum, silicon, zirconium and/or transition metal oxide and/or hydroxide.

TPER

The term "transition metal" is intended to mean more particularly the metals of the fourth period ranging from scandium to zinc, provided they are, of course, compatible in terms of innocuity with the intended application. It is more particularly a titanium, manganese, iron, cobalt, nickel or copper oxide or hydroxide.

Of course, this inorganic shell can comprise oxides and/or hydroxides of different natures.

Silicon, aluminum, titanium and zirconium oxides and/or hydroxides are more particularly suitable for the present invention.

According to a preferential mode of the invention, the capsules comprise an inorganic shell based on at least one silicon oxide.

In general, the size of the capsules according to the invention can be between 1 and several tens of micrometers. The size of the particles of the inorganic substance constituting the shell of these capsules can, itself, vary between 1 and 200 nm.

With regard more particularly to the thickness of the inorganic shell, it can vary between 1 and 200 nm.

These capsules can also be characterized by the amount of liquid medium which the inorganic shell retains, by introducing the capsule retention parameter. This corresponds to the ratio of the mass of the liquid medium contained in the capsule and that of the inorganic shell.

When the shell is too porous, the capsule retention is low. It is of which possible to vary the capsule retention by setting the size of the particles made of the material constituting the inorganic shell, and also the thickness of the latter.

A subject of the present invention is also a process which can be used for preparing inorganic capsules in accordance with the present invention, said process comprising

1) emulsifying a liquid medium containing at least one active biological substance, in a second phase which is immiscible with said liquid medium, so as to disperse it therein in the form of droplets,

2) bringing into contact, in the inverse emulsion thus obtained, at least one hydrolyzable and polycondensable zirconium, silicon, aluminum and/or transition metal compound, under temperature and pH

conditions conducive to the formation of a precipitate consisting of the corresponding oxide or hydroxide, and

3) recovering the inorganic capsules thus formed and, where appropriate, purifying them,

said process being characterized in that the formation of the inorganic precipitate in the second step is carried out in the presence of an amphiphilic surfactant system present in the emulsion and capable of concentrating the deposit of the inorganic particles of said precipitate formed at the interface of the droplets and the second phase, and of effectively blocking their diffusion in said droplets.

The amphiphilic surfactant system provided according to the invention has the advantage of blocking the phenomenon of natural diffusion of the inorganic particles towards the center of the droplets.

For the purpose of the invention, the term "amphiphilic surfactant system" is intended to refer to either a single compound in which two regions coexist, which two regions have very different solubilities and are sufficiently distant from each other so as to behave independently, or a combination of at least two compounds having very different solubilities, such as a first compound which is hydrophilic in nature and a second compound which is hydrophobic in nature. Generally, these two regions or compounds comprise,

respectively, at least one hydrophilic group and one or more long chains which are hydrophobic in nature.

Consequently, the surfactant system used according to the invention can be represented by a single compound which will then be introduced prior to carrying out the second step, i.e. the hydrolysis and polycondensation step, or resulted from the interaction, in situ, of at least two compounds, such as for example an organosoluble surfactant initially present in the generally organosoluble second phase and a water-soluble compound present in the generally aqueous liquid medium. It is also possible to envisage coupling or complexing between a first organosoluble agent and a second organosoluble agent ionic in nature, such as a quaternary ammonium. The two compounds meet at the interface of the droplets formed during the emulsifying. By virtue of their interaction, they contribute, on the one hand, toward stabilizing the system by decreasing the interfacial tension at the interface of the droplets, and probably act as a steric or electrostatic barrier.

In the process claimed, the embodiment using at least two distinct compounds capable of interacting so as to produce a surfactant system able to effectively block the diffusion of the inorganic particles in the aqueous droplets and to stabilize said emulsion is more particularly preferred.

The surfactant system provided according to the invention preferably comprises at least one surfactant with an HLB value of less than 7.

The term "HLB" refers to the ratio of the hydrophilicity of the polar groups of the surfactant molecule to the hydrophobicity of the lipophilic portion of this same molecule.

In this particular case, the two compounds are preferably present in the generally aqueous liquid medium and the generally organosoluble second phase, respectively, and interact with each other when the liquid medium is emulsified in said second phase.

This option has the advantage of conferring satisfactory stability on the corresponding emulsion as soon as it is formed. Furthermore, it proves possible, if necessary, by suitably selecting the agents constituting the amphiphilic surfactant system, to adjust the pH to a value compatible with the active substance.

With regard to the emulsifying, it can be carried out by applying intense mechanical stirring energy to the two initial phases, and/or sonication. The size of the droplets obtained at the end of the emulsifying step can be between approximately 0.1 and ten or so µm.

The compound present in the generally aqueous liquid medium preferably has viscosity-modifying action.

More particularly, this compound can be a compound chosen from sugars and derivatives thereof. Oses (or monosaccharides), osides and highly depolymerized polyholosides are suitable in this respect. Compounds with a molar mass by weight of more particularly less than 20 000 g/mol are intended.

Among the oses, mention may be made of aldoses such as glucose, mannose or galactose, and ketoses such as fructose.

Osides are compounds which result from the condensation, with removal of water, of ose molecules with non-carbohydrate molecules. Among the osides, preference is given to holosides which are formed by the coming together of exclusively carbohydrate motifs, and more particularly, oligoholosides (or oligosaccharides) which include only a limited number of these motifs, i.e. a number generally less than or equal to 10. By way of examples of oligoholosides, mention may be made of sucrose, lactose, cellobiose and maltose.

The suitable highly depolymerized polyholosides (or polysaccharides) are described, for example, in the work by P. ARNAUD entitled "Cours de chimie organique [Organic Chemistry Course]", editors

GUTHIER-VILLARS, 1987. More particularly, polyholosides with a molecular mass by weight of more particularly less than 20 000 g/mol are used.

By way of nonlimiting example of highly depolymerized polyholosides, mention may be made of dextran, starch, xanthan gum and galactomannans such as guar or carob. These polysaccharides preferably have a melting weight above 100°C and a water-solubility of between 10 and 500 g/l.

Also suitable for the invention are gum arabic, gelatin and fatty derivatives thereof such as fatty acid sucroesters, carbohydrate alcohols of the sorbitol or mannitol type, carbohydrate ethers such as methyl, ethyl, carboxymethyl, hydroxyethyl and hydroxypropyl ethers of cellulose, and glycerols, pentaerythrol, propylene glycol, ethylene glycol, nonviscous diols and/or polyvinyl alcohols.

It is preferably a hydrocolloid. By way of representation of this type of compound, mention may be made in particular of alginates, polysaccharides of the natural gum type, such as carrageenans, xanthan and guar, and most particularly cellulose derivatives.

Preferably, it is a cellulose derivative, and more preferably hydroxyethylcellulose.

The generally organosoluble surfactant(s) present in the second phase can be chosen from fatty alcohols, triglycerides, fatty acids, sorbitan esters,

fatty amines, these compounds possibly being in a polyalkoxylated form, liposoluble lecithins, polyalkylene dipolyhydroxystearates, quaternary ammonium salts, monoglycerides, polyglyceryl esters, polyglyceryl polyricinoleate and lactic acid esters.

The fatty alcohols generally comprise from 6 to 22 carbon atoms. The triglycerides can be triglycerides of plant or animal origin (such as lard, tallow, groundnut oil, butter oil, cottonseed oil, flax oil, olive oil, fish oil, copra oil or coconut oil).

The fatty acids are fatty acid esters (such as for example oleic acid or stearic acid).

The sorbitan esters are cyclized fatty acid esters of sorbitol comprising from 10 to 20 carbon atoms, such as lauric acid, stearic acid or oleic acid.

According to a preferred mode of the invention, this surfactant is a sorbitan ester as defined above, and more preferably sorbitan sesquioleate.

As emerges from the presentation above, the compound present in the generally aqueous liquid medium must interact with the surfactant present in the generally hydrophobic second phase so as to produce a surfactant system capable of constituting an effective diffusion barrier with respect to the particles of the inorganic precipitate. Consequently, the respective

choices thereof must be made while taking this requirement into account.

Of course, the nature of the active substance to be encapsulated, as well as the composition of the inorganic shell of the capsules prepared according to the invention, are also elements which are determinant in choosing the surfactant system and assessing the respective amounts of the corresponding two compounds. These adjustments in fact fall within the competence of those skilled in the art.

In the particular case in which, according to the invention, the use of a single compound of amphiphilic type is favored, the compounds most particularly suitable are those satisfying the general formula I:

$$R_2-(A)_n-[\underset{\underset{B}{|}}{N}-(CHR_1)_x]_y-\underset{\underset{B}{|}}{N}-Q \qquad \text{I}$$

in which:

R_2 represents an alkyl or alkenyl radical comprising 7 to 22 carbon atoms, R_1 represents a hydrogen atom or an alkyl radical comprising 1 to 6 carbon atoms, A represents a (CO) or (OCH_2CH_2) group, n has a value of 0 or 1, x has a value of 2 or 3, y has a value of 0 to 4, Q represents an R_3-COOM radical with R_2 representing an alkyl radical comprising 1 to 6 carbon atoms, M represents a hydrogen atom, an alkali

where is M?

metal, an alkaline-earth metal or a quaternary ammonium group in which the radicals linked to the nitrogen atom, which may be identical or different, are chosen from hydrogen or an alkyl or hydroxyalkyl radical having 1 to 6 carbon atoms, and B represents H or Q.

Preferably, M represents a hydrogen atom, sodium, potassium or an NH_4 group.

Among these surfactants corresponding to formula I, use is more particularly made of the amphoteric derivatives of alkylpolyamines, such as amphionic XL®, Mirataine H2C-HA®, sold by Rhodia Chimie, and also Ampholac 7T/X®, sold by Berol Nobel.

It is also possible to use a nonionic main surfactant, the hydrophilic portion of which contains one or more saccharide motif(s). Said saccharide motifs generally contain from 5 to 6 carbon atoms. They can derive from sugars such as fructose, glucose, mannose, galactose, talose, gulose, allose, altose, idose, arabinose, xylose, lyxose and/or ribose.

Among these surfactants having a saccharide structure, mention may be made of alkylpolyglycosides. They can be obtained by condensation (for example by acid catalysis) of glucose with primary fatty alcohols (US-A-3 598 865; US-A-4 565 647; EP-A-132 043; EP-A-132 046; Tenside Surf. Det. 28, 419, 1991, 3; Langmuir 1993, 9, 3375-3384) having a C_4-C_{20} alkyl group, preferably of the order of 1.1 to 1.8 per mole

of alkylpolyglycoside (APG); mention may be made in particular of those sold, respectively, under the names GLUCOPON 600 EC®, GLUCOPON 650 EC® and GLUCOPON 225 CSUP®, by HENKEL.

By way of illustration, the concentration of amphiphilic surfactant system can be between approximately 1% and 10% by weight with respect to the organosoluble phase.

According to a preferential mode of the invention, the surfactant incorporated into the generally organosoluble second phase is a sorbitan ester, and more preferably sorbitan sesquioleate. With regard to the compound incorporated into the liquid medium, it is preferably a cellulose derivative, and more particularly hydroxyethylcellulose.

According to a preferred embodiment of the invention, the substance of the inorganic shell derives from the hydrolysis and polycondensation of one or more alkoxides of formula II.

$$M(R)_n(P)_m \qquad II$$

in which:

- M represents an element chosen from titanium, manganese, iron, cobalt, nickel, silicon, aluminum and zirconium,
- R is a hydrolyzable substituent,
- n is an integer between 1 and 6,
- P is a nonhydrolyzable substituent, and

- m is an integer between 0 and 6.

According to a preferred mode of the invention:

- M is chosen from silica, aluminum, titanium and zirconium,
- R is a group chosen from C_1 to C_{18}, and preferably C_2 to C_8, alkoxy and/or aryloxy groups, and n is an integer between 2 and 4, and
- P is a group chosen from C_1 to C_8 alkyl, C_2 to C_8 aryl or C_2 to C_8 alkenyl groups.

With regard to R, it is preferably a C_1 to C_6, and more preferably C_2 to C_4, alkoxy group. This alkoxy group can, where appropriate, be substituted with a C_1 to C_4 alkyl or alkoxy group, or a halogen atom. In general formula II, R can represent alkoxy groups which may be identical or different.

Of course, several compounds of formula II can be used.

As discussed above, it proves possible, through the choice of this hydrolyzable and polycondensable inorganic compound, to adjust the porosity and size of the capsules.

Similarly, it is possible to confer a more or less hydrophobic nature on the capsule by adjusting the nature and, for example, the length of the alkyl and/or alkoxy chains making up this hydrolyzable and polycondensable inorganic compound.

Generally, the hydrolysis and polycondensation of this inorganic precursor are either accomplished spontaneously by mixing this precursor together with the emulsion, or are initiated by adjusting the pH and/or the temperature of the emulsion to a value conducive to them taking place. This adjustment may, in particular, arise from the presence in the emulsion of water-soluble ions such as NH_4OH, NaOH or HCl, or organosoluble ions of amine type. These adjustments fall within the competence of those skilled in the art.

According to a preferred variant of the present invention, the inorganic shell obtained according to the invention is based on silicon oxide. It derives from the precipitation of at least one silicate.

As a silicate which is suitable for the present invention, mention may be made more particularly of tetramethyl orthosilicate, TMOS, tetraethyl orthosilicate, TEOS, tetrapropyl orthosilicate, TPOS, alkylalkoxysilanes and haloalkylsilanes.

According to a particular embodiment of the invention, the inorganic capsule is obtained by formation of an inorganic precipitate in the presence of an agent for hydrolyzing and condensing said compound.

The hydrolysis of these silicon alkoxides can take place both by acid catalysis and by basic catalysis, on condition that the corresponding oxides and/or hydroxides are obtained in a pulverulent form.

Preferably, a silicon alkoxide such as tetraethylorthosilicate, TEOS, in the presence of aqueous ammonia, is used as a hydrolysis and polycondensation agent.

The second phase is generally an oily phase which is immiscible with the generally aqueous liquid medium and is preferably composed of an oil chosen from plant, animal and inorganic oils. It can, for example, be a paraffin oil or a silicone oil.

However, it is also possible to envisage using other organic solvents, such as perfluoro solvents, on condition that these solvents are used under suitable conditions, for example in the form of a mixture so as to produce an emulsion with the liquid medium.

As a second phase which is most particularly suitable for the invention, mention may be made in particular of the solvent Isopar®, which is an isoparaffin sold by the company Exxon Chemicals.

This second phase comprises at least one generally organosoluble surfactant which is preferable chosen from sorbitan esters, and more preferably is represented by sorbitan sesquioleate.

With regard to the generally aqueous liquid medium, it comprises at least one hydrocolloid, optionally the agent for hydrolyzing and polycondensing the hydrolyzable and polycondensable inorganic precursor.

With regard to the hydrocolloid, it is preferably a cellulose derivative, and more preferably hydroxyethylcellulose.

The inorganic capsules according to the invention are particularly advantageous for uses in the fermentation, biomedical and food domains and in the chemical industry.

It is thus possible to envisage immobilizing, in the capsules claimed, cells having an activity which is advantageous for the production of pharmaceutical products, of metabolites and/or of reagents such as intermediates of chemical or pharmaceutical synthesis, or of biodegradable polymers.

The immobilization according to the invention of microroganisms such as yeasts or bacteria is also particularly advantageous for the food industry, and most particularly for the dairy and wine industries.

Enzymes immobilized according to the invention may, themselves, represent reagents of choice in many industrial manufacturing processes, for catalytic or analytical methods.

Similarly, it is possible to envisage using capsules according to the invention based on living cells or on enzymes, in the treatment of waste water or of waste.

The examples and the figure which follow are given by way of nonlimiting illustration of the subject of the present invention.

Figures:

- Figure 1: Scanning microscopy (SEM) photograph of capsules according to the invention incorporating E. coli.
- Figure 2: Visualization of encapsulated E. coli by transmission electron microscopy (TEM).

Raw materials:

Isopar M® (EXXON)	density at 15°C: 0.786
Arlacel 83® (ICI)	sorbitan sesquioleate
HEC (SIGMA ALDRICH)	hydroxyethylcellulose
Aqueous ammonia (NH_4OH solution	density at 20°C: 0.880 NH_3 concentration: 20%
Methyl silicate: $Si(OMe)_4$	molar mass: 156 g density at 20°C: 1.032

Sodium phosphate buffer at pH 7.

Escherichia Coli K12 expressing a β-galactosidase.

Example 1: Encapsulation of E. Coli

Overall composition of the reaction medium:

Aqueous phase:	H_2O	43.40 g	
	HEC	2.61 g	(6%/water)

NH_3	5.00 g	(1 mol/1)
E. coli	4 g	(i.e. 1 g of dry cells)

Organic phase:	Arlacel 83®	17.35 g
	Isopar M®	850 g
tetramethylorthosilane®		TMOS 28.5 g

Preparation of the aqueous phase:

The HEC is homogenized in purified water in a waterbath at 40°C for approximately 20 minutes. A clear yellow viscous mixture is thus obtained. The cells and then the aqueous ammonia solution are then added thereto.

Preparation of the organic phase:

The Arcacel 83® is solubilized in the Isopar M®.

Preparation of the emulsion:

The organic phase and the aqueous phase are emulsified using an Ultraturrax®, and an oil/water emulsion having a stability of several hours is thus obtained. The size of the droplets is close to ten or so microns.

Synthesis of the capsules:

The emulsion prepared above is introduced into a 2-liter three-necked flask equipped with a magnetic stirrer bar.

The tetramethyl orthosilane, TMOS, (28.5 g) is added thereto with a flow rate of 0.5 ml/min (duration of the introduction of TMOS = 1 hour) at 25°C.

The particles obtained are separated and washed with methanol then twice with phosphate buffer, by centrifugation, and then dried at room temperature overnight.

<u>Characterization</u>:

The size of the particles of silica which constitutes the shell is in the region of a few nanometers.

The capsules are about ten or so µm in size (SEM). The encapsulation of the bacteria is perfectly visualized by TEM (transmission electron miscrocopy), as is the porosity of the capsule.

The photographs given in figures 1 and 2 show the appearance of these capsules.

<u>Example 2: Determination of the enzymatic activity of the biocapsules</u>

The enzymatic activity of the E. Coli β-galactosidase is determined after enzymatic hydrolysis of para-nitrophenyl-β-D-galactoside (p-NPG) to p-nitrophenol. The quantitative measurement of the p-nitrophenol by spectrophotometry makes it possible to deduce therefrom the enzymatic activity of the biocapsules.

The enzymatic activity of the biocapsules of example 1 is expressed in µmol/h/mg of DC (dry cells), this being in comparison to the bioactivity of the starting cells. The enzymatic activity of the starting cells is 0.2 µmol/h/mg of DC, the biocapsule activity yield is approximately 50%. The activity of the cells is therefore well preserved.

Example 3: Production of silica capsules, with the particles making up the shell being a few nanometers in size.

Overall composition of the reaction mixture:

Aqueous phase:	H_2O	43.40 g	
	HEC	2.61 g	(6%/water)
	NH_3	5.0 g	(1 mol/l)
	E. coli	4 g	(i.e. 1 g of dry cells)

Organic phase:	Arlacel 83®	17.35 g	(2.04%/Isopar)
	Isopar M®	850 g	
	TEOS	39 g	

Preparation of the aqueous phase:

The HEC is homogenized in purified water in a waterbath at 40°C for approximately 20 minutes. A clear yellow viscous mixture is thus obtained. The cells and then the aqueous ammonia solution are then added thereto.

Preparation of the organic phase:

The Arcacel 83® is solubilized in the Isopar M®.

Preparation of the emulsion:

The organic phase and the aqueous phase are emulsified using an Ultraturrax, and an oil/water dispersion having a stability of several hours is thus obtained. The size of the droplets is close to ten or so microns.

Synthesis of the capsules:

The emulsion prepared above is introduced into a 2-liter three-necked flask equipped with a magnetic stirrer bar.

The ethyl silicate (39 g) is added thereto with a flow rate of 0.8 ml/min (duration of the introduction of TEOS = 1 hour) at 25°C.

The capsules obtained are separated and washed once with ethanol then twice with the phosphate buffer, and then dried at room temperature overnight.

Characterization:

The capsules are between 1 and ten or so μm in size (SEM).

The particles of silica constituting the shell are a few nanometers in size. The observations made by TEM clearly demonstrate the encapsulation of the bacteria by the silica capsule.

Example 4: Production of capsules with TMOS and co-alkoxide ($[NH_3]$ = 0.1 mol/l).

Overall composition of the reaction medium:

Aqueous phase:	H_2O	43.40 g
	HEC	2.61 g (6%/water)
	NH_3	0.37 g i.e. $[NH_3]$ = 0.1 mol/l
	E. coli	4 g (i.e. 1 g of dry cells)

Organic phase:	Arlacel 83®	17.35 g (2.04%/Isopar)
	Isopar M®	850 g

Organic phase 2:	TMOS	22.8 g
	O-TMOS	5.7 g

Preparation of the aqueous phase:

The HEC is homogenized in purified water in a waterbath at 40°C for approximately 20 minutes. A clear yellow viscous mixture is thus obtained. The cells and then the aqueous ammonia solution (0.37 g) are then added thereto.

Preparation of organic phase 1:

The Arcacel 83® is solubilized in the Isopar M®.

Preparation of the emulsion:

Organic phase 1 and the aqueous phase are emulsified using an Ultraturrax, and an oil/water dispersion having a stability of several hours is thus

obtained. The size of the droplets is close to ten or so microns.

Procedure:

The emulsion prepared above is introduced into a 2-liter three-necked flask equipped with a magnetic stirrer bar.

The mixture of alkoxides (organic phase 2) is added thereto with a flow rate of 0.5 ml/min (the duration of the introduction is 1 hour) at 25°C.

The particles obtained are separated and washed with methanol and then dried at room temperature overnight. The particles thus obtained are hydrophobic.

Characterization:

The particles are between 1 and ten or so µm in size (SEM).

The particles of silica constituting the shell are a few nanometers in size (TEM).

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A process for preparing an inorganic capsule consisting of an inorganic shell and of a liquid core in which at least one active biological substance is immobilized, said process comprising

a) emulsifying a liquid medium containing at least one active biological substance, in a second phase which is immiscible with said liquid medium, so as to disperse it therein in the form of droplets,

b) bringing into contact, in the emulsion thus obtained, a hydrolyzable and polycondensable silicon, under temperature and pH conditions conducive to the formation of a precipitate consisting of the corresponding oxide or hydroxide, and

c) recovering the inorganic capsules thus formed and, where appropriate, purifying them, wherein the formation of the inorganic precipitate in the second step is carried out in the presence of an amphiphilic surfactant system present in the emulsion and capable of concentrating the deposit of the inorganic particles of said precipitate formed at the interface of the droplets and the second phase, and of effectively blocking their diffusion in said droplets.

2. A process for preparing an inorganic capsule consisting of an inorganic shell and of a liquid core in which at least one active biological substance is immobilized, said process comprising

a) emulsifying a liquid medium containing at least one active biological substance, in a second phase which is immiscible with said liquid medium, so as to disperse it therein in the form of droplets,

b) bringing into contact, in the emulsion thus obtained, at least one hydrolyzable and polycondesable zirconium, aluminium and/or transition metal compound, under temperature and pH conditions conducive to the formation of a precipitate consisting of the corresponding oxide or hydroxide, and

c) recovering the inorganic capsules thus formed and, where appropriate, purifying them, wherein the formation of the inorganic precipitate in the second step is carried out in the presence of an amphiphilic surfactant system present in the emulsion and capable of concentrating the deposit of the inorganic particles of said precipitate formed at the interface of the droplets and the second phase, and of effectively blocking their diffusion in said droplets.

3. The process as claimed in claim 1 or claim 2, wherein the amphiphilic surfactant system results from the interaction, in situ, between a first compound present in the second phase and a compound present in the liquid medium.

4. The process as claimed in any one of claims 1 to 3, wherein the surfactant system comprises

at least one surfactant with an HLB value of less than 7.

5. The process as claimed in any one of claims 1 to 4, wherein the surfactant present in the second phase is a sorbitan ester.

6. The process as claimed in claim 3, wherein the compound present in the liquid medium is a cellulose derivative.

7. The process as claimed in any one of claims 1 to 6, wherein the emulsion is obtained mechanically and/or by sonication.

8. The process as claimed in claim 1, wherein the hydrolyzable and polycondensable compound satisfies general formula II:

$$M(R)_n(P)_m \qquad II$$

in which:

- M represents silicon,
- R is a hydrolyzable substituent,
- n is an integer between 1 and 6,
- P is a nonhydrolyzable substituent, and
- m is an integer between 0 and 6.

9. The process as claimed in claim 2, wherein the hydrolyzable and polycondensable compound satisfies general formula II:

$$M(R)_n(P)_m \qquad II$$

in which:

- M represents an element chosen from titanium, manganese, iron, cobalt, nickel, aluminium and zirconium,
- R is a hydrolyzable substituent,
- n is an integer between 1 and 6,
- P is a nonhydrolyzable substituent, and
- m is an integer between 0 and 6.

10. The process as claimed in claim 8, wherein the hydrolyzable and polycondensable compound is a silicon alkoxide.

11. The process as claimed in claim 10, wherein the silicon alkoxide is chosen from tetramethylorthosilicate, tetraethylorthosilicate and tetrapropylorthosilicate.

12. The process as claimed in any one of claims 1 to 11, wherein the formation of the precipitate is carried out in the presence of an agent for hydrolyzing and condensing said compound.

13. The process as claimed in claim 12, wherein the hydrolysis and polycondensation agent is aqueous ammonia.

14. A process for preparing an inorganic capsule, substantially as herein described with reference to any one of the embodiments of the invention as illustrated in the accompanying drawings and/or examples but excluding comparative examples.

15. An inorganic capsule consisting of an inorganic shell and of a liquid core, in which at least one active biological substance is immobilized, said inorganic capsule being prepared by a process according to any one of the preceding claims.

16. Use of the inorganic capsule as claimed in claim 15, in the fermentation, biomedical and food domains, and in the chemical industry.

DATED this 27th day of May 2003

BALDWIN SHELSTON WATERS

Attorneys for: RHODIA CHIMIE

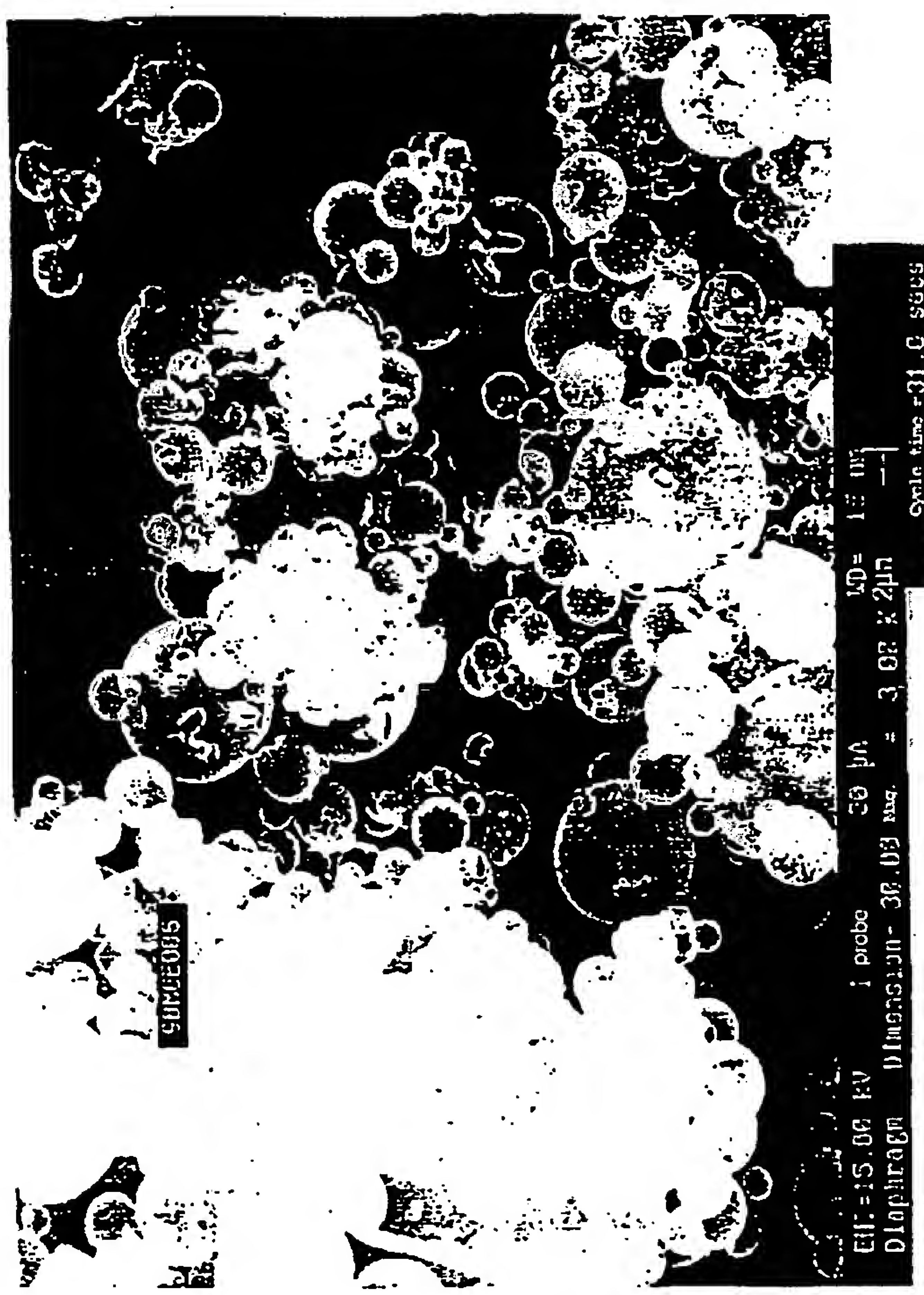

FIG.1

FIG 1

FIG. 2